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Klotho sensitivity of the hERG channel

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ABSTRACT

Klotho, a hormone and enzyme, is a powerful regulator of ageing and life span. Klotho deficiency leads to cardiac arrythmia and sudden cardiac death. We thus explored whether klotho modifies cardiac K^+-channel hERG. Current was determined utilizing dual electrode voltage clamp and hERG protein abundance utilizing immunohistochemistry and chemiluminescence in *Xenopus* oocytes expressing hERG with or without klotho. Coexpression of klotho increased cell membrane hERG-protein abundance and hERG current at any given voltage without significantly modifying the voltage required to activate the channel. The effect of klotho coexpression was mimicked by recombinant klotho protein and reversed by β-glucuronidase-inhibitor D-saccharic acid-1,4-lactone.

1. Introduction

Klotho, a protein expressed in several tissues including kidney, parathyroid glands and choroid plexus [1,2], has a profound influence on ageing and life span [3,4]. Klotho deficient mice suffer from severe growth retardation as well as accelerated appearance of a wide variety of age related disorders and eventually die within less than 5 months [4]. Conversely, the life span of klotho-overexpressing mice is substantially prolonged [3,4]. The extracellular domain of klotho may be cleaved off and influence target cells by its action as glucuronidase or hormone [5].

Klotho participates in the inhibitory effect of FGF23 on 1α-hydroxylase resulting in decreased formation of $1,25(OH)_2D_3$ [1,3,6,7]. Since $1,25(OH)_2D_3$ is a powerful regulator of intestinal and renal Ca^{2+} and phosphate transport [8,9], the excessive $1,25(OH)_2D_3$ formation in klotho deficient mice results in a marked increase of plasma Ca^{2+} [10] and phosphate [9] concentrations with subsequent vascular calcification [11], growth deficit [1] and premature erythrocyte death [12].

Klotho is further known to modify ion channels, carriers and pumps in the cellular plasma membrane, including Na^+, phosphate cotransporters [13,14], Na^+/K^+ ATPase [15], Ca^{2+} channels [16] and renal outer medullary K^+ channels [17]. Klotho insufficiency results in cardiac arrhythmia and sudden cardiac death [2], which could potentially have resulted from deranged klotho-dependent regulation of cardiac ion channels.

The present study thus explored whether klotho participates in the regulation of the human ether-a-go-go (hERG) channel, a key channel accomplishing cardiac repolarisation [18,19] and participating in the deranged excitation following cardiac hypertrophy [20]. hERG is further known to foster proliferation of several tumour cells [21–23]. In order to possibly disclose an influence of klotho on hERG channels, hERG was expressed in *Xenopus* oocytes with or without additional expression of klotho. The results reveal that coexpression of klotho up-regulates hERG activity by enhancing the plasma membrane abundance of the channel protein. The effect is mimicked by treatment of hERG expressing *Xenopus* oocytes with recombinant klotho protein. Conversely, the current in hERG and klotho expressing *Xenopus* oocytes is down-regulated by treatment with β-glucuronidase-inhibitor D-saccharic acid-1,4-lactone (DSAL).

2. Materials and methods

2.1. Experiments in Xenopus oocytes

For generation of cRNA, constructs were used encoding klotho [13] and hERG [24]. The cRNA was generated as described previously [25]. For voltage clamp analysis, *Xenopus* oocytes were prepared as previously described [26]. Oocytes were injected with water and 7.5 ng cRNA encoding hERG with and without 10 ng cRNA encoding klotho. Standard two electrode voltage clamp

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recordings were performed 3 days after hERG injection [27]. The oocytes were maintained at 17 °C in a solution containing (in mM) 88.5 NaCl, 2 KCl, 1.8 $CaC1_2$, 1 $MgC1_2$, 5 HEPES, 0.11 tretracycline, 0.004 ciprofloxacin, 0.22 gentamycin (Refobacin ©), 0.5 theophylline (Euphylong ©) as well as 5 sodium pyruvate. The pH was adjusted to 7.4 by addition of NaOH. Oocytes were superfused continuously with ND-96 buffer containing (mM): NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1 and HEPES 5 (pH 7.4 with NaOH). Pipettes were filled with 3 M KCl and had resistances of 0.5–1.0 MΩ. The data were filtered at 1 kHz and the experiments were performed with a Geneclamp 500 amplifier (Axon Instruments, Union City, CA, USA) and a Digidata 1322A interface (Axon Instruments, Union City, CA, USA) [28]. Data acquisition was achieved with pCLAMP 9.02 (Axon Instruments, Union City, CA, USA) and the data analysis was performed with Clampfit 9.2 (Axon Instruments) software. Where indicated, the experiments were performed in *Xenopus* oocytes treated with either, recombinant klotho protein (24 h, 30 ng/ml) or D-saccharic acid-1,4-lactone (DSAL, 24 h, 10 μM). Leak currents estimated from the tail current measured after the preconditioning prepulse to −80 mV were subtracted. For normalization, the individual tail currents at +70 mV were divided by the mean tail current at +70 mV of oocytes expressing hERG alone.

2.2. Detection of hERG surface expression by chemiluminescence

For detection of hERG cell surface expression by chemiluminescence, the oocytes were first incubated with 1 μg/ml primary rat monoclonal anti-HA antibody (clone 3 F10, Roche, Mannheim, Germany) and subsequently with secondary, HRP-conjugated goat anti-rat IgG antibody (1:1000, Cell Signaling Technology, MA, USA). Individual oocytes were placed in 96 well plates with 20 μl of SuperSignal ELISA Femto Maximum Sensitivity Substrate (Pierce, Rockford, IL, USA) and chemiluminescence of single oocytes was quantified in a luminometer (Walter Wallac 2 plate reader, Perkin Elmer, Juegesheim, Germany) by integrating the signal over a period of 1 s [29]. Results display relative light units. Integrity of the measured oocytes was assessed by visual control after the measurement to avoid unspecific light signals from the cytosol.

2.3. Immunostaining and confocal microscopy

To visualize hERG-HA cell surface expression, oocytes were fixed in 4% paraformaldehyde/PBS for at least 2 h at room temperature [30]. After washing with PBS, the oocytes were cryoprotected in 30% sucrose, frozen in mounting medium and placed on a cryostat. Sections were collected at a thickness of 8 μm on coated slides and stored at −20 °C. For immunostaining, sections were thawed at room temperature, fixed in aceton/methanol (1:1), washed in PBS and blocked for 1 h in 5% bovine serum albumin in PBS. Sections were incubated overnight at 4 °C with 1 μg/ml primary rat monoclonal anti-HA antibody (clone 3 F10, Roche, Mannheim, Germany). Binding of primary antibody was visualised with fluorescence-labelled secondary Alexa Fluor 488 anti-rat IgG (1:200, Invitrogen, UK) for 1 h at room temperature. The slides were mounted with

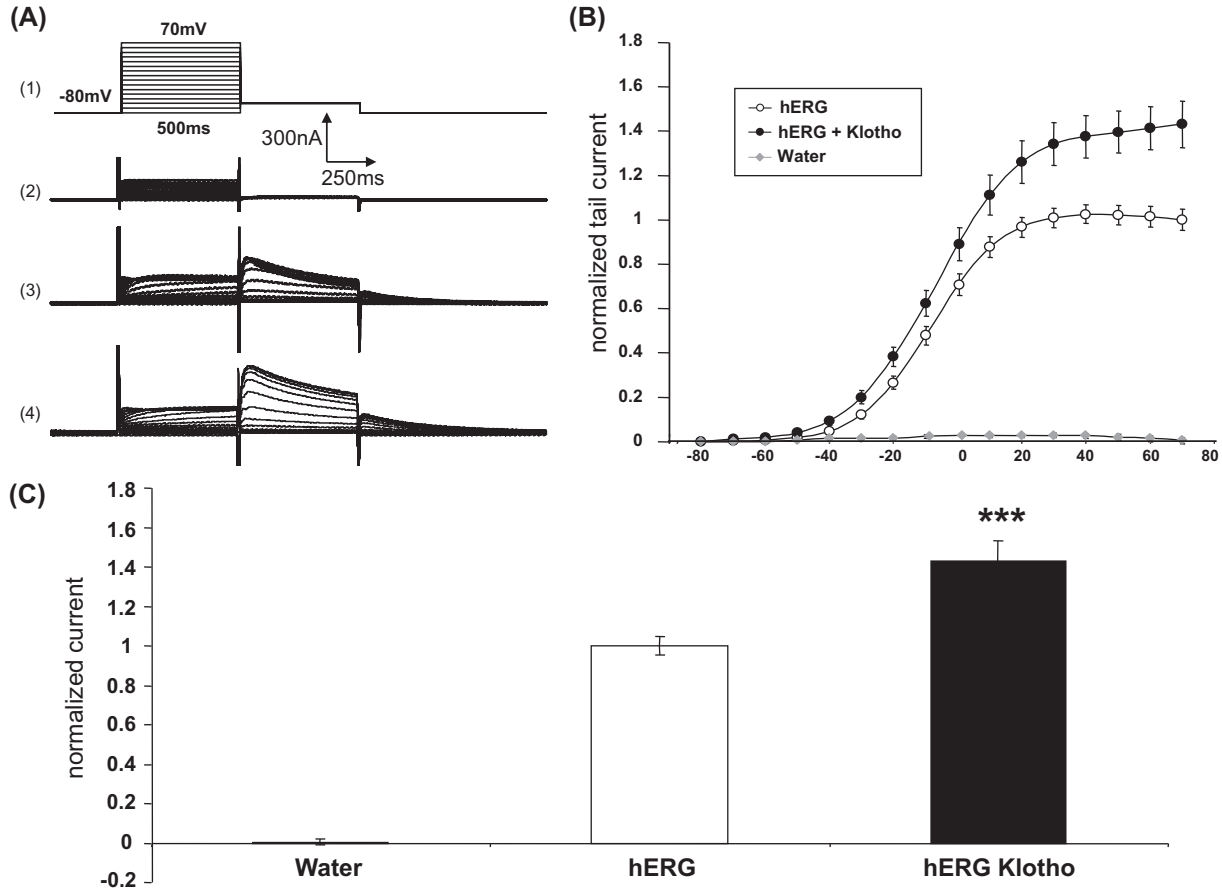

Fig. 1. Effect of klotho coexpression on current in hERG expressing *Xenopus* oocytes. (A) Original tracings recorded in oocytes injected with water (2), with cRNA encoding hERG alone (3) or coexpressing hERG and klotho (4). The pulse protocol is illustrated in (1): The oocytes were depolarized from a holding potential of −80 mV to different voltages followed by a 500 ms pulse to −60 mV evoking outward tail currents. (B) Arithmetic means ± S.E.M. (n = 19–37) of the normalized leak-corrected peak tail current as a function of voltage in oocytes injected with water (grey diamonds), with cRNA encoding hERG (white circles) or coexpressing hERG and klotho (black circles). (C) Arithmetic means ± S.E.M. (n = 19–37) of the normalized tail current following a depolarization to +70 mV recorded in oocytes injected with water (dotted bar), with cRNA encoding hERG (white bar) or with cRNA encoding hERG and klotho (black bar). *** indicates statistical significant (P < 0.001) difference to oocytes expressing hERG alone.

Pro Long Gold antifade reagent (Invitrogen, UK). Images were taken on a Zeiss LSM 510 EXCITER Confocal Laser Scanning Microscope (Carl Zeiss MicroImaging GmbH, Germany) with A-Plan 40×/1.2 W. Brightness and contrast settings were kept constant during imaging of all oocytes in each injection series.

2.4. Statistical analysis

Data are provided as arithmetic means ± S.E.M; *n* represents the number of oocytes or cells investigated. All oocyte experiments were repeated with at least three batches of oocytes; in all repetitions, qualitatively similar data were obtained. All data were tested for significance by using ANOVA. Results with $P < 0.05$ were considered statistically significant.

3. Results

In order to test whether klotho modifies the current generated by hERG channels, cRNA encoding hERG was injected into *Xenopus* oocytes with or without cRNA encoding klotho. As illustrated in Fig. 1, in hERG expressing but not in water-injected *Xenopus* oocytes depolarisations (500 ms) from a holding potential of −80 mV followed by repolarizations to −60 mV evoked characteristic hERG outward tail currents. The additional expression of klotho resulted in a marked increase of the hERG tail current. Normalization of the tail currents to the maximum peak tail current of the respective group reveals that coexpression of klotho did not significantly modify the voltage required to activate the channels. The voltage of half maximal peak tail current was similar in *Xenopus* oocytes expressing hERG alone and in *Xenopus* oocytes expressing hERG with klotho.

The effect of klotho could have resulted from an increase of hERG protein abundance in the cell membrane. Confocal microscopy and chemiluminescence were thus employed to quantify hERG protein abundance in the cell membrane. To this end, HA-tagged hERG was expressed in *Xenopus* oocytes and binding of HA-specific antibody visualized by confocal microscopy and quantified by chemiluminescence (Fig. 2). Due to unspecific staining, considerable chemiluminescence was observed even in water injected oocytes and the expression of hERG led only to a moderate increase of HA-dependent staining in confocal microscopy and increase of chemiluminescence. Nevertheless, the coexpression of klotho resulted in a significant increase of hERG channel protein abundance in the plasma membrane (Fig. 2).

Further experiments were performed to test whether the effect of klotho expression could be mimicked by treatment of hERG expressing *Xenopus* oocytes with recombinant klotho protein. As illustrated in Fig. 3, the treatment of hERG expressing *Xenopus* oocytes with klotho protein for 24 h was followed by a significant increase of the hERG induced tail current. Similar to what has been observed following coexpressing klotho in hERG expressing *Xenopus* oocytes, the treatment of hERG expressing *Xenopus* oocytes with klotho protein increased the maximal peak current values but did not significantly modify the voltage required for activation of hERG channels.

As klotho is known to be effective as a glucuronidase [5] modifying the stability of proteins in the cell membrane [31], the effect of the klotho inhibitor DSAL was tested on tail currents in *Xenopus* oocytes expressing both, hERG and klotho. As illustrated in Fig. 4, the treatment of hERG and klotho expressing *Xenopus* oocytes with the klotho inhibitor d-saccharic acid-1,4-lactone (DSAL, 10 μM) for a one day period significantly decreased the absolute peak current

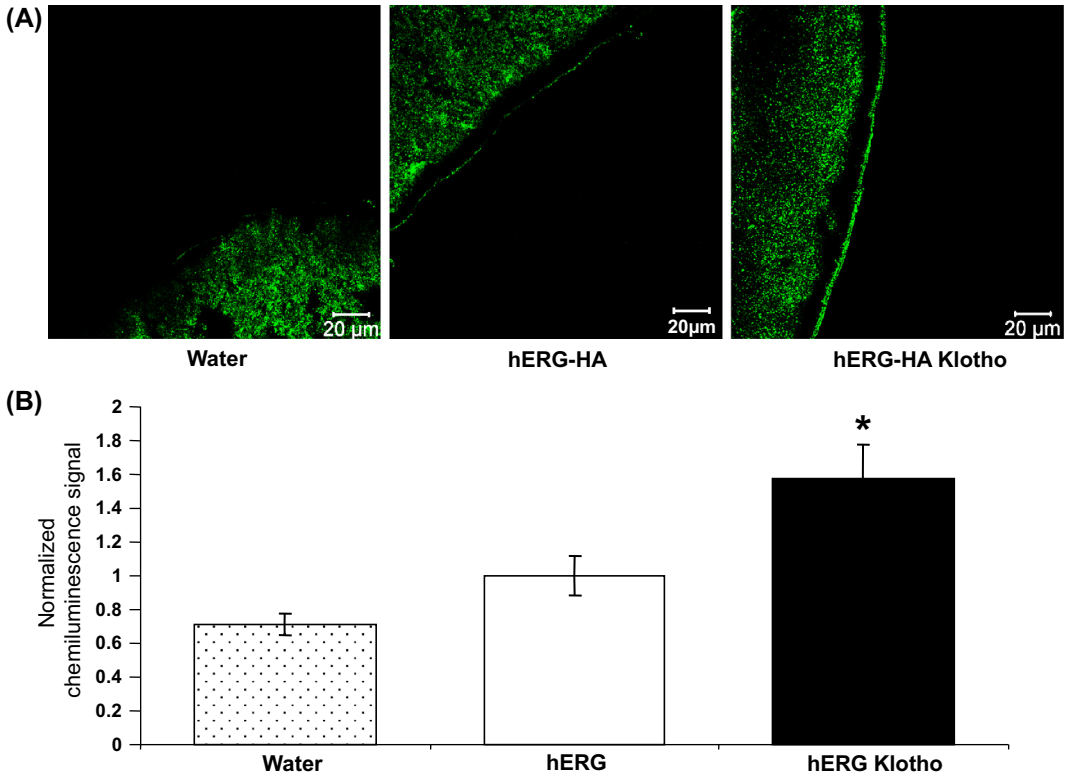

Fig. 2. Effect of klotho coexpression on hERG protein abundance in the cell membrane of *Xenopus* oocytes. (A) Confocal microscopy of hERG protein abundance in the plasma membrane of *Xenopus* oocytes injected with water (left panel) or expressing hERG without (middle panel) or with (right panel) treatment with klotho. The cells were subjected to immunofluorescence staining using a FITC-conjugated antibody (green). (B) Arithmetic means ± S.E.M. (n = 35–63) of hERG protein abundance as determined by chemiluminescence in the plasma membrane of *Xenopus* oocytes injected with water (dotted bar) or expressing hERG without (white bar) or with (black bar) additional expression of klotho. * indicates statistical significant ($p<0.05$) difference from oocytes expressing hERG-HA alone.

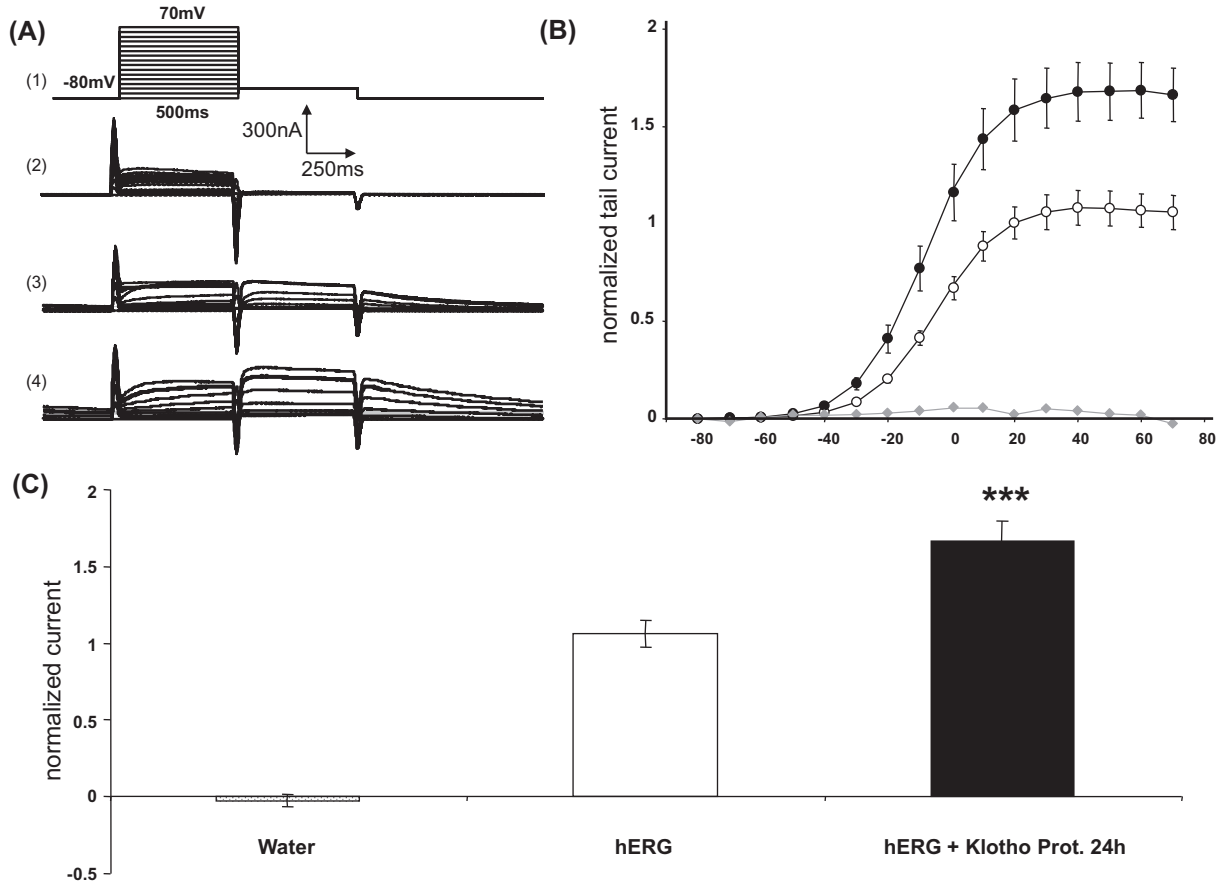

Fig. 3. Effect of treatment with klotho protein on current in hERG expressing *Xenopus* oocytes. (A) Original tracings recorded in oocytes injected with water (2), expressing hERG without treatment (3) or treated for 24 h (4) with human recombinant klotho protein (30 ng/ml). The pulse protocol is illustrated in (1): the oocytes were depolarized from a holding potential of −80 mV to different voltages followed by a 500 ms pulse to −60 mV evoking outward tail currents. (B) Arithmetic means ± S.E.M. (n = 9–18) of the normalized leak-corrected peak tail current as a function of voltage in oocytes injected with water (grey diamonds), or injected with cRNA encoding hERG without (white circles) or with (black circles) treatment for 24 h with human recombinant klotho protein. (C) Arithmetic means ± S.E.M. (n = 9–18) of the normalized tail current following a depolarization to +70 mV recorded in water injected oocytes (dotted bar), or in hERG expressing oocytes without treatment (white bar) or treated (black bars) with recombinant klotho protein (24 h, 30 ng/ml). *** indicates statistical significant (P < 0.001) difference from oocytes expressing untreated hERG.

values without significantly modifying the voltage sensitivity of hERG channels.

4. Discussion

The present observations reveal a novel function of klotho, i.e., the regulation of hERG channel activity. According to dual electrode voltage clamp in hERG channel expressing *Xenopus* oocytes, klotho significantly increased the hERG protein abundance within the cell membrane. The effect was mimicked by treatment of hERG expressing oocytes with recombinant klotho protein.

The effect of klotho on hERG channels may result at least in part from its enzyme activity. Klotho has glucuronidase activity [5]. Klotho has been shown to up-regulate epithelial Ca^{2+} channels TRPV5 [16,32,33] and the renal outer medullary K^+ channel ROMK [17] by its enzyme activity. It has been suggested that klotho cleaves the terminal sialic acids from N-glycan chains of the channel proteins thus exposing the underlying disaccharide galactose-*N*-acetylglucosamine [34]. The galactose-*N*-acetylglucosamine binds galectin-1, which prevents internalization thus leading to accumulation of the channels in the plasma membrane [34,34]. Moreover, full-length klotho has a chaperone-like effect on surface-membrane expression of channels [35–37].

The effect of klotho on hERG protein abundance in the cell membrane and thus on hERG channel activity presumably influences the cardiac action potential. An increase of hERG activity is expected to accelerate the repolarisation of ventricular cardiomyocytes and shorten the action potential. Klotho deficiency is expected to down-regulate hERG channel activity resulting in delayed repolarisation. Whether or not the cardiac arrhythmia and sudden cardiac death in klotho deficiency [2] is caused by deranged regulation of ion channel activity, remains to be shown.

hERG channels are down-regulated in cardiac hypertrophy [20,38], an effect mediated by activation of AT_1 receptors with subsequent activation of protein kinase C linked to the PKC pathway in ventricular cardiomyocytes [39]. It may be of interest whether treatment with klotho could reverse the down-regulation of hERG channel activity in cardiac hypertrophy.

hERG channels are further known to facilitate tumour growth [21–23]. At least in theory, the up-regulation of hERG by klotho could thus foster tumour development.

In conclusion, klotho participates in the regulation of the voltage-gated K^+ channel hERG. Klotho enhances hERG activity, an effect apparently requiring glucuronidase activity.

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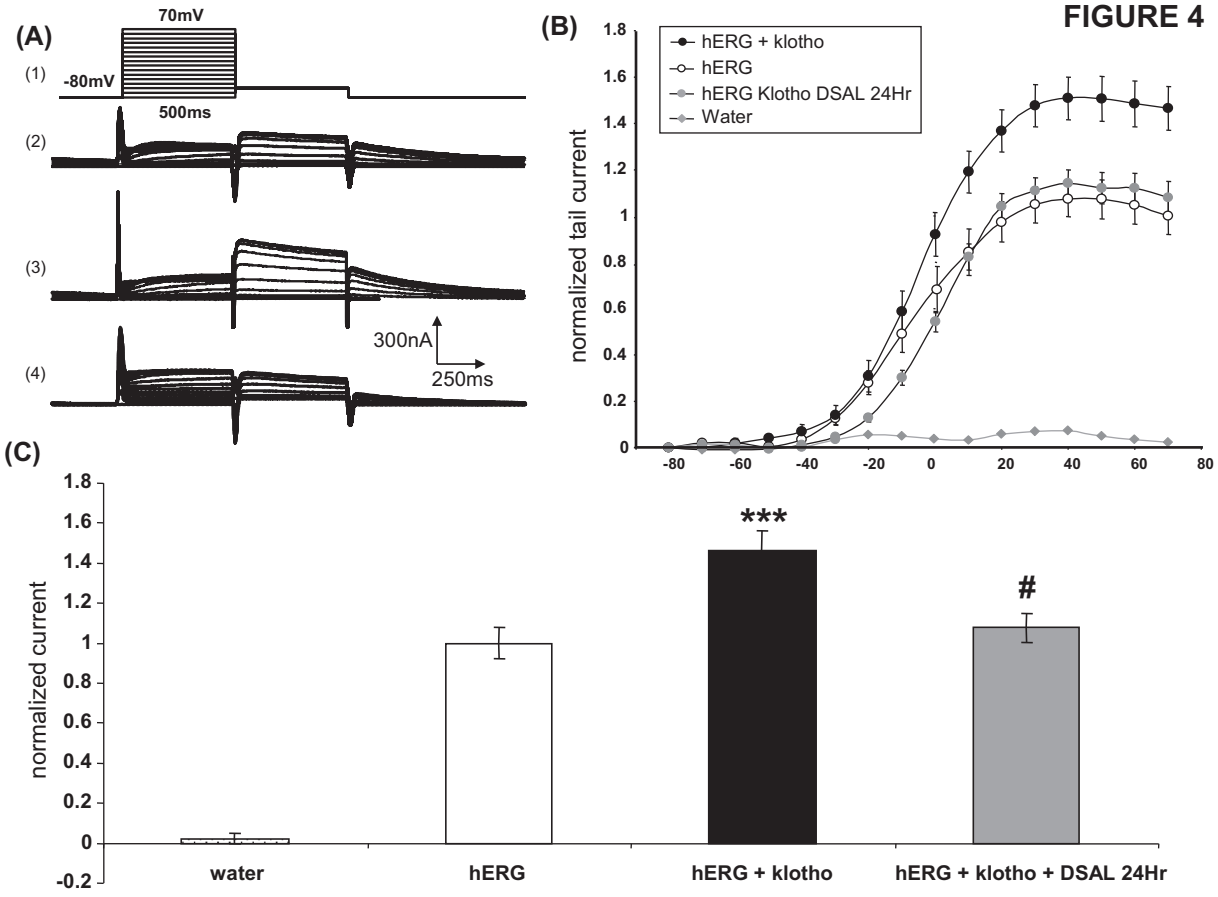

Fig. 4. Effect of klotho inhibitor DSAL on current in hERG and klotho expressing *Xenopus* oocytes. (A) Original tracings recorded in oocytes expressing hERG without (2) or with klotho without (3) or with (4) a 24 h pretreatment with klotho inhibitor DSAL (10 μM). The pulse protocol is illustrated in (1): the oocytes were depolarized from a holding potential of −80 mV to different voltages followed by a 500 ms pulse to −60 mV evoking outward tail currents. (B) Arithmetic means ± S.E.M. (n = 6–12) of the normalized leak-corrected peak tail current as a function of voltage in oocytes injected with water (grey diamonds), or expressing hERG alone (white circles) or expressing hERG and klotho without treatment (black circles) or treated for 24 h with klotho inhibitor DSAL (grey circles). (C) Arithmetic means ± S.E.M. (n = 6–12) of the normalized tail current following a depolarization to +70 mV recorded in water injected oocytes (dotted bar), expressing hERG alone (white bar) or expressing hERG with klotho without treatment (black bar) or with (grey bar) pretreatment with d-saccharic acid-1,4-lactone (DSAL, 24 h 10 μM). *** indicates statistical significant ($P < 0.001$) difference from oocytes expressing hERG alone. # indicates statistical significant ($p<0.05$) difference from oocytes co-expressing hERG and Klotho without DSAL treatment.

References

[1] Tsujikawa, H., Kurotaki, Y., Fujimori, T., Fukuda, K. and Nabeshima, Y. (2003) Klotho, a gene related to a syndrome resembling human premature aging, functions in a negative regulatory circuit of vitamin D endocrine system. Mol. Endocrinol. 17, 2393–2403.

[2] Takeshita, K., Fujimori, T., Kurotaki, Y., Honjo, H., Tsujikawa, H., Yasui, K., Lee, J.K., Kamiya, K., Kitaichi, K., Yamamoto, K., Ito, M., Kondo, T., Iino, S., Inden, Y., Hirai, M., Murohara, T., Kodama, I. and Nabeshima, Y. (2004) Sinoatrial node dysfunction and early unexpected death of mice with a defect of klotho gene expression. Circulation 109, 1776–1782.

[3] Kuro-o, M. (2010) Klotho. Pflugers Arch. 459, 333–343.

[4] Kuro-o, M., Matsumura, Y., Aizawa, H., Kawaguchi, H., Suga, T., Utsugi, T., Ohyama, Y., Kurabayashi, M., Kaname, T., Kume, E., Iwasaki, H., Iida, A., Shiraki-Iida, T., Nishikawa, S., Nagai, R. and Nabeshima, Y.I. (1997) Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 390, 45–51.

[5] Kuro-o, M. (2012) Klotho in health and disease. Curr. Opin. Nephrol. Hypertens. 21, 362–368.

[6] Razzaque, M.S., Sitara, D., Taguchi, T., St. Arnaud, R. and Lanske, B. (2006) Premature aging-like phenotype in fibroblast growth factor 23 null mice is a vitamin D-mediated process. FASEB J. 20, 720–722.

[7] Yoshida, T., Fujimori, T. and Nabeshima, Y. (2002) Mediation of unusually high concentrations of 1,25-dihydroxyvitamin D in homozygous klotho mutant mice by increased expression of renal 1alpha-hydroxylase gene. Endocrinology 143, 683–689.

[8] Ramasamy, I. (2006) Recent advances in physiological calcium homeostasis. Clin. Chem. Lab. Med. 44, 237–273.

[9] Segawa, H., Yamanaka, S., Ohno, Y., Onitsuka, A., Shiozawa, K., Aranami, F., Furutani, J., Tomoe, Y., Ito, M., Kuwahata, M., Imura, A., Nabeshima, Y. and Miyamoto, K. (2007) Correlation between hyperphosphatemia and type II Na-Pi cotransporter activity in klotho mice. Am. J. Physiol. Renal Physiol. 292, F769–F779.

[10] Kuro-o, M. (2006) Klotho as a regulator of fibroblast growth factor signaling and phosphate/calcium metabolism. Curr. Opin. Nephrol. Hypertens. 15, 437–441.

[11] Ohnishi, M., Nakatani, T., Lanske, B. and Razzaque, M.S. (2009) Reversal of mineral ion homeostasis and soft-tissue calcification of klotho knockout mice by deletion of vitamin D 1alpha-hydroxylase. Kidney Int. 75, 1166–1172.

[12] Kempe, D.S., Ackermann, T.F., Fischer, S.S., Koka, S., Boini, K.M., Mahmud, H., Foller, M., Rosenblatt, K.P., Kuro, O. and Lang, F. (2009) Accelerated suicidal erythrocyte death in Klotho-deficient mice. Pflugers Arch. 458, 503–512.

[13] Dermaku-Sopjani, M., Sopjani, M., Saxena, A., Shojaiefard, M., Bogatikov, E., Alesutan, I., Eichenmuller, M. and Lang, F. (2011) Downregulation of NaPi-IIa and NaPi-IIb Na-coupled phosphate transporters by coexpression of Klotho. Cell. Physiol. Biochem. 28, 251–258.

[14] Hu, M.C., Shi, M., Zhang, J., Pastor, J., Nakatani, T., Lanske, B., Razzaque, M.S., Rosenblatt, K.P., Baum, M.G., Kuro-o, M. and Moe, O.W. (2010) Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. FASEB J. 24, 3438–3450.

[15] Sopjani, M., Alesutan, I., Dermaku-Sopjani, M., Gu, S., Zelenak, C., Munoz, C., Velic, A., Foller, M., Rosenblatt, K.P., Kuro-o, M. and Lang, F. (2011) Regulation of the Na^+/K^+ ATPase by Klotho. FEBS Lett. 585, 1759–1764.

[16] Boros, S., Bindels, R.J. and Hoenderop, J.G. (2009) Active Ca(2+) reabsorption in the connecting tubule. Pflugers Arch. 458, 99–109.

[17] Cha, S.K., Hu, M.C., Kurosu, H., Kuro-o, M., Moe, O. and Huang, C.L. (2009) Regulation of renal outer medullary potassium channel and renal K(+) excretion by Klotho. Mol. Pharmacol. 76, 38–46.

[18] Sanguinetti, M.C. (1999) Dysfunction of delayed rectifier potassium channels in an inherited cardiac arrhythmia. Ann. N.Y. Acad. Sci. 868, 406–413.

[19] Vincent, G.M. (1998) The molecular genetics of the long QT syndrome: genes causing fainting and sudden death. Annu. Rev. Med. 49, 263–274.

[20] Wang, J., Wang, H., Zhang, Y., Gao, H., Nattel, S. and Wang, Z. (2004) Impairment of HERG K(+) channel function by tumor necrosis factor-alpha: role of reactive oxygen species as a mediator. J. Biol. Chem. 279, 13289–13292.

[21] Arcangeli, A. (2005) Expression and role of hERG channels in cancer cells. Novartis Found. Symp. 266, 225–232.
[22] Asher, V., Sowter, H., Shaw, R., Bali, A. and Khan, R. (2010) Eag and HERG potassium channels as novel therapeutic targets in cancer. World J. Surg. Oncol. 8, 113.
[23] Pillozzi, S. and Arcangeli, A. (2010) Physical and functional interaction between integrins and hERG1 channels in cancer cells. Adv. Exp. Med. Biol. 674, 55–67.
[24] Dong, Z.X., Zhao, X., Gu, D.F., Shi, Y.Q., Zhang, J., Hu, X.X., Hu, M.Q., Yang, B.F. and Li, B.X. (2012) Comparative effects of liensinine and neferine on the human ether-a-go-go-related gene potassium channel and pharmacological activity analysis. Cell. Physiol. Biochem. 29, 431–442.
[25] Mia, S., Munoz, C., Pakladok, T., Siraskar, G., Voelkl, J., Alesutan, I. and Lang, F. (2012) Downregulation of Kv1.5 K channels by the AMP-activated protein kinase. Cell. Physiol. Biochem. 30, 1039–1050.
[26] Henrion, U., Zumhagen, S., Steinke, K., Strutz-Seebohm, N., Stallmeyer, B., Lang, F., Schulze-Bahr, E. and Seebohm, G. (2012) Overlapping cardiac phenotype associated with a familial mutation in the voltage sensor of the KCNQ1 channel. Cell. Physiol. Biochem. 29, 809–818.
[27] Pathare, G., Foller, M., Daryadel, A., Mutig, K., Bogatikov, E., Fajol, A., Almilaji, A., Michael, D., Stange, G., Voelkl, J., Wagner, C.A., Bachmann, S. and Lang, F. (2012) OSR1-sensitive renal tubular phosphate reabsorption. Kidney Blood Press Res. 36, 149–161.
[28] Strutz-Seebohm, N., Pusch, M., Wolf, S., Stoll, R., Tapken, D., Gerwert, K., Attali, B. and Seebohm, G. (2011) Structural basis of slow activation gating in the cardiac I Ks channel complex. Cell. Physiol. Biochem. 27, 443–452.
[29] Hosseinzadeh, Z., Bhavsar, S.K. and Lang, F. (2012) Down-regulation of the myoinositol transporter SMIT by JAK2. Cell. Physiol. Biochem. 30, 1473–1480.
[30] Hosseinzadeh, Z., Bhavsar, S.K., Sopjani, M., Alesutan, I., Saxena, A., Dermaku-Sopjani, M. and Lang, F. (2011) Regulation of the glutamate transporters by JAK2. Cell. Physiol. Biochem. 28, 693–702.
[31] Imura, A., Iwano, A., Tohyama, O., Tsuji, Y., Nozaki, K., Hashimoto, N., Fujimori, T. and Nabeshima, Y. (2004) Secreted Klotho protein in sera and CSF: implication for post-translational cleavage in release of Klotho protein from cell membrane. FEBS Lett. 565, 143–147.
[32] Cha, S.K., Ortega, B., Kurosu, H., Rosenblatt, K.P., Kuro, O. and Huang, C.L. (2008) Removal of sialic acid involving Klotho causes cell-surface retention of TRPV5 channel via binding to galectin-1. Proc. Natl. Acad. Sci. USA 105, 9805–9810.
[33] Chang, Q., Hoefs, S., van der Kemp, A.W., Topala, C.N., Bindels, R.J. and Hoenderop, J.G. (2005) The beta-glucuronidase klotho hydrolyzes and activates the TRPV5 channel. Science 310, 490–493.
[34] Huang, C.L. (2012) Regulation of ion channels by secreted Klotho. Adv. Exp. Med. Biol. 728, 100–106.
[35] Bankston, J.R., Camp, S.S., DiMaio, F., Lewis, A.S., Chetkovich, D.M. and Zagotta, W.N. (2012) Structure and stoichiometry of an accessory subunit TRIP8b interaction with hyperpolarization-activated cyclic nucleotide-gated channels. Proc. Natl. Acad. Sci. USA 109, 7899–7904.
[36] Yu, H., Wu, J., Potapova, I., Wymore, R.T., Holmes, B., Zuckerman, J., Pan, Z., Wang, H., Shi, W., Robinson, R.B., El Maghrabi, M.R., Benjamin, W., Dixon, J., McKinnon, D., Cohen, I.S. and Wymore, R. (2001) MinK-related peptide 1: a beta subunit for the HCN ion channel subunit family enhances expression and speeds activation. Circ. Res. 88, E84–E87.
[37] Gong, Q., Anderson, C.L., January, C.T. and Zhou, Z. (2002) Role of glycosylation in cell surface expression and stability of HERG potassium channels. Am. J. Physiol. Heart Circ. Physiol. 283, H77–H84.
[38] Hu, C., Yan, C., Lin, J., Liu, S. and Li, Y. (2011) Down-regulation of the human ether-a-go-go-related gene in rat cardiac hypertrophy. Am. J. Med. Sci. 341, 119–125.
[39] Wang, Y.H., Shi, C.X., Dong, F., Sheng, J.W. and Xu, Y.F. (2008) Inhibition of the rapid component of the delayed rectifier potassium current in ventricular myocytes by angiotensin II via the AT1 receptor. Br. J. Pharmacol. 154, 429–439.